

Phthalate Ester Metabolism in the Isolated, Perfused Rat Liver System

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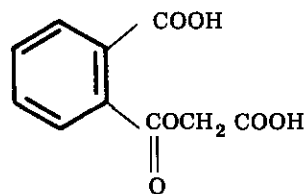
A series of experiments, performed at Johns Hopkins University, which led to our finding that plasticizers are extracted from poly-(vinyl chloride) (PVC) plastic by blood and biologic fluids will be described.

The experiments began with the appearance of an unknown compound in the isolated perfused liver system. Livers were perfused with a mixture of synthetic plasma containing bovine serum albumin and rat blood. At the end of the perfusion the livers were homogenized, a perchloric acid extract made, and column chromatography performed. In one such experiment, in addition to the expected adenine nucleotides, an additional peak was observed which eluted from the column between ADP and ATP. The unidentified material absorbed ultra-violet light at 260 nm. A comparable peak was not observed when samples of liver were assayed prior to perfusion. In addition to this finding of an unidentified material in the perfused liver, we also observed accumulation of this material in the perfusate. This result is shown in Figure 1.

In this figure the broken lines indicate the acid-soluble extract obtained from perfusion fluid that was not circulated through an intact liver; the solid lines indicate the compounds that appear in the presence of

the liver. The lines labeled AMP and ADP represent amounts of radioactive nucleotides which were added to the extract to serve as chromatographic markers. It can be seen from Figure 1 that the peak labeled 1 is common to both experimental conditions. Peaks 2 and 3 appeared only when an intact liver was in the system. Peak 3 was the same as the unidentified material found in samples of perfused liver. Thus the appearance of the unidentified material depended on the presence of an intact liver and perfusion of that liver within the apparatus.

Instrumental analysis was performed. The information gained from these analyses led to the conclusion that the molecule contained an aromatic diacid portion, a single esterified grouping, and a free acid function. The most likely combination of C, H, and O that fits these various criteria is glycolyl phthalate (GP), which is an aromatic diacid (phthalic acid) and an ester (glycolic acid).



Glycolyl Phthalate (GP)

The presence of a phthalate compound in the perfusate of the isolated rat liver was indeed a strange finding. These chemicals are not usual constituents of living organisms, but it is well known that esters of phthalic

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acid are used as plasticizers of poly (vinyl chloride) tubing and containers. It was possible that the appearance of glycolyl phthalate might have resulted from contamination of the perfusate by a plasticizer from the PVC tubing used in the perfusion apparatus. Subsequent metabolism of the plasticizer by the isolated perfused liver might

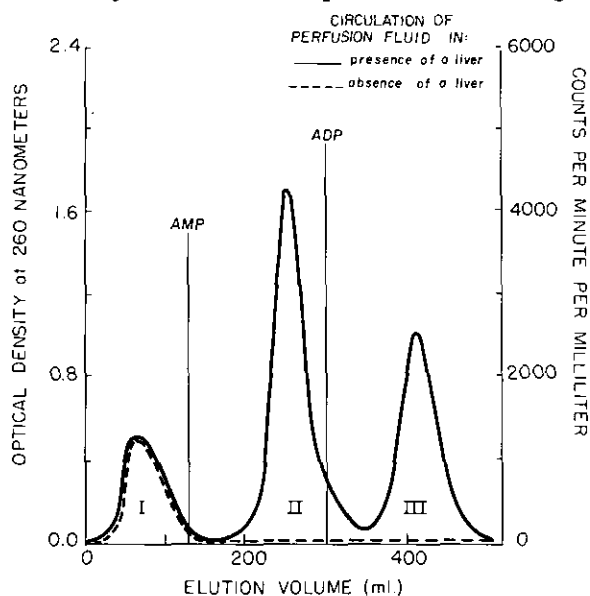


FIGURE 1. Circulation of perfusion fluid in (—) the presence of and (---) the absence of a liver. The perfusion fluid, a mixture of 70 ml of whole rat blood containing 70 units of heparin per milliliter and 35 ml of Krebs-Ringer bicarbonate buffer containing 4% BSA and 80 mg glucose/100 ml, was circulated in a liver perfusion apparatus. This system was used to perfuse an isolated rat liver for 4 hr, or the perfusion fluid was allowed to circulate in the absence of a liver for the same length of time. At the end of the experiment, the total plasma was isolated by centrifugation and acidified with perchloric acid. After removal of the precipitate, the acid-soluble supernatant was neutralized with KOH and centrifuged and the supernatant recovered. An amount of ^{14}C -adenosine diphosphate and ^{14}C -adenosine monophosphate was added to the neutralized extract to act as a marker during further chromatographic fractionation. The total extract was applied to a 0.7×10 cm column of Dowex-1 (formate form) anion-exchange resin. Elution of the column was with a nonlinear gradient of ammonium formate (0–2N, pH 5.5), and the absorbance at 260 nm was monitored continuously and radioactivity measured in a Packard TriCarb liquid scintillation counter. Only the Peak radioactive fractions are shown in this figure (1).

have led to GP. The tubing manufacturer confirmed that the plasticizer used in his tubing was butylglycolyl butyl phthalate and supplied us with authentic samples of the pure material.

We confirmed the origin of glycolyl phthalate by doing a series of perfusion experiments. The perfusion system was assembled with the glass apparatus normally employed but in one experiment the PVC tubing was replaced by ether-washed latex tubing. The results of this series of experi-

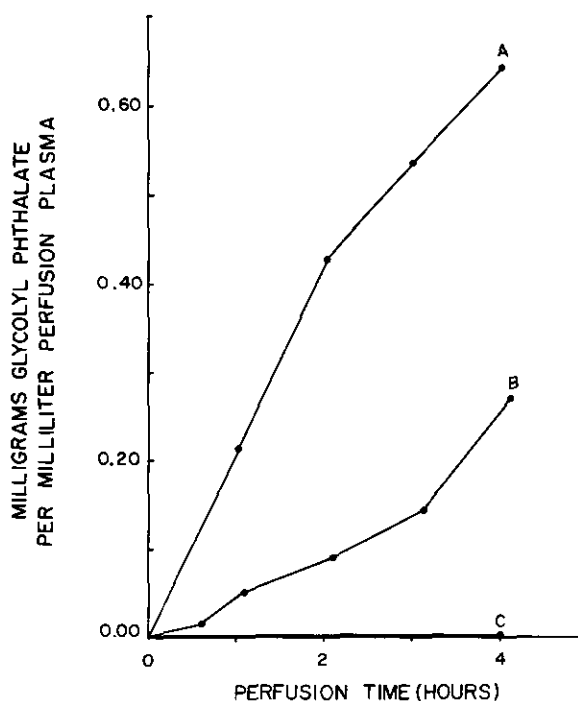


FIGURE 2. Perfusion experiments. The content of glycolyl phthalate was determined as follows. In experiment A, after extraction of the plasma with chloroform at neutral pH to remove lipid-soluble material, a portion of the remaining aqueous phase was fractionated by thin-layer chromatography. The spot corresponding to GP was scraped from the plate, the GP was eluted from the powder into water, and its concentration was determined by its ultraviolet absorption. In experiment B, the plasma sample was acid-precipitated. The increase in absorbance at 280 nm was taken as a measure of the amount of GP accumulating. In experiment C, only the 4-hr point was determined. This was done by fractionating the acid-soluble supernatant fraction from the total perfusate on a Dowex-1 column. As indicated, no GP was detected (1).

ments are shown in Figure 2. It can be seen that the perfusion with latex tubing alone, perfusion C, produced no glycolyl phthalate. However, the perfusion which employed the normal amount of PVC tubing produced some glycolyl phthalate (perfusion B). When 600 mg of PG butylglycolyl butyl phthalate was added directly to the perfusate and allowed to circulate through the liver, the amount of glycolyl phthalate represented by line A accumulated. Thus we had shown

that the unknown compound was a metabolite of a phthalate ester plasticizer extracted from the PVC tubing of our perfusion system. This, then, is the beginning of our observations that plasticizers may be extracted from PVC plastic.

REFERENCE

1. Jaeger, R.J., and Rubin, R.J. 1970. Plasticizers from plastic devices: extraction, metabolism, and accumulation by biological systems. *Science* 170: 460.